AD

TECHNICAL REPORT
NATICK/TR-81/003

LEVEL II

FLUOROMETRIC METHOD FOR DETERMINATION OF URIC ACID IN FLOUR

by
L. G. Holmes
and
L. W. Smith, Jr.

Approved for public release;
distribution unlimited.

July 1980

UNITED STATES ARMY
NATICK RESEARCH and DEVELOPMENT LABORATORIES
NATICK, MASSACHUSETTS 01760

Food Sciences Laboratory
FSL

Approved for public release; distribution unlimited.

Citation of trade names in this report does not constitute an official indorsement or approval of the use of such items.

Destroy this report when no longer needed. Do not return it to the originator.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: NATICK/TR-81/003	2. GOVT ACCESSION NO.: AD-A100 657
3. RECIPIENT'S CATALOG NUMBER:	
4. TITLE (and Subtitle): FLUOROMETRIC METHOD FOR DETERMINATION OF URIC ACID IN FLOUR.	5. TYPE OF REPORT & PERIOD COVERED: Final
	6. PERFORMING ORG. REPORT NUMBER:
7. AUTHOR(s): L. G. Holmes and L. W. Smith, Jr.	8. CONTRACT OR GRANT NUMBER(s): 16
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Food Sciences Laboratory, U.S. Army Natick R&D Laboratories, Natick, MA 01760	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 62724A, IL162724AH99, BE, 001
11. CONTROLLING OFFICE NAME AND ADDRESS: Food Sciences Laboratory (DRDNA-YMB), U.S. Army Natick R&D Laboratories, Natick, MA 01760	12. REPORT DATE: 11 July 1980
	13. NUMBER OF PAGES: 14
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office): 15 C2	15. SECURITY CLASS. (of this report): Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE:

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

Service Requirement Identification: DLA 6-1, Research on Insect Infestation of Food

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Flour	Ultraviolet Spectroscopy
Uric Acid	Insects
Insect Infestation	Fluorometry
Fluorescence Spectroscopy	Tribolium

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A fluorescence technique is described for the determination of uric acid content in flour and its use as an index of insect infestation. Uric acid, a major component of the excreta of adult Tribolium used in this study for natural infestation, accumulates in infested products providing a quantitative measure of past plus present degree of infestation. The efficiency of uric acid extraction from flour with 0.2 M sodium acetate, checked by adding known amounts of uric acid to flour, is shown to be about 96-98% accurate. The method is capable of detection of uric acid at the level of 3.0 mg per 100 g of flour.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Preface

This study was undertaken by the Food Biochemistry Group, Food Sciences Laboratory, U.S. Army Natick Research and Development Laboratories, Natick, Massachusetts, in response to Defense Logistics Agency Requirement 6-1, Research on Insect Infestation of Food.

The authors wish to acknowledge the contributions of student aides, Marc Bystock, Food Engineering Laboratory and Paula M. Chinetti, Food Sciences Laboratory, for their valuable technical assistance.

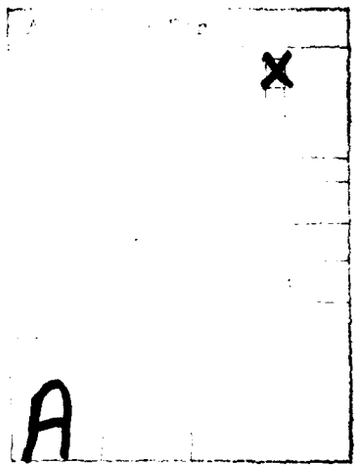

Table of Contents

List of Illustrations

FLUOROMETRIC METHOD FOR DETERMINATION OF URIC ACID IN FLOUR

Introduction

Chemical and microanalytical techniques have been devised for determining the degree of insect infestation of flours; however, the present techniques have certain limitations. A chemical technique in use as a regulatory method for the determination of the degree of insect infestation in stored flour is the enzymatic-ultraviolet absorption assay for determining uric acid content.[1] Approximately 18% of the total excreta from Tribolium confusum is uric acid.[2] Various investigators have established good agreement in the correlation of degrees of infestation with uric acid content of flour.[3,4,5,6] Although the spectrophotometric assay has good specificity, it is a somewhat lengthy analytical technique. In addition, turbidity, incomplete enzyme action, and interfering coloration at high pH may also arise further complicating this analytical technique. A microanalytical technique widely used for the determination of the degree of insect infestation of flour is the counting of the number of insects fragments. The method, however, would not be applicable for flour apparently free from insects in which prior evidence had been removed and would therefore not be detectable by the fragment-counting method. Kurtz and Harris have summarized a number of papers which describe this technique.[7]

Fluorescence spectroscopy, closely related to the widely used technique based on ultraviolet-visible spectroscopy, has assumed an important role in analytical applications. Due to enhanced specificity over conventional spectroscopy, the use of fluorometry has long been a valuable method of analysis. In addition to specificity, fluorometric methods are simple and

---

[1]Association of Official Analytical Chemists, 1980. Official Methods of Analysis (13th ed.), Method 44:178. The Association, Washington, DC.

[2]P. D. Gupta and R. N. Sinha, 1960. Ann. Entomol. Soc. Am., 53:632.

[3]S. Ven Katrao, R. N. Nuggehalli, S. V. Pringle, M. Swaminathan and V. Subrahmanyan, 1960. Cereal Chem., 37:97.

[4]G. Farn and D. M. Smith, 1963. J. Assoc. Off. Anal. Chem., 46:522.

[5]N. P. Sen and D. M. Smith, 1966. J. Assoc. Off. Anal. Chem., 49:899.

[6]N. P. Sen, 1968. J. Assoc. Off. Anal. Chem., 51:785.

[7]O. L. Kurtz and K. L. Harris, 1962. Micro-Analytical Entomology for Sanitation Control, Assoc. Off. Agric. Chem., Washington, DC, p. 13.

accurate, allowing laboratory findings to be more easily translated to simplified inspection methods and devices. Simplified methods are required so that laboratory personnel and/or inspectors can evaluate with confidence the utility of stored stocks of flour. The results of this investigation suggest the applicability of the fluorometric technique as an analytical method for the determination of uric acid content in flour and its use as an index of insect infestation which may be correlated with serviceability.

Materials and Methods

Two species of insects, Tribolium confusum (Du Val) and Tribolium castaneum (Hbst.) were reared separately in a culture medium prepared according to the method described by Harein and Soderstrom.[8]

The adult insects used for testing purposes were removed from the culture medium and housed in 500 ml widemouth glass containers with filter paper inserts in the open metal covers. One hundred g of hard wheat flour were weighed into each of the containers prior to the introduction of the insects. Two test units, each consisting of three 500 ml containers, were infested with T. confusum and T. castaneum, respectively, at levels of 20, 40, and 60 insects. Two uninfested containers served as controls. All containers were kept in environmental chambers at 25°C and 55% relative humidity. Three samples from each level of insect infestation including the controls were tested at 4, 6, and 8-day intervals. Insects were removed from the flour prior to analysis by sifting through a 50-mesh screen.

A standard curve was prepared for uric acid. One hundred mg of accurately weighed uric acid were dissolved in 100 ml of 0.2-M sodium acetate buffer adjusted to pH 11.8-12.0 with 2N NaOH. Dilutions of the stock uric acid standard were prepared volumetrically with the acetate buffer to give 4.0, 12.0, 20.0, 28.0, and 48.0-mg/dL solutions. Fluorescence was measured on a fluorometer (Hitachi Model MPF-2A). An excitation wavelength of 330 nm, using a 390-nm filter for the emission monochromator, gave an emission maximum at 420-nm. Emission maxima versus uric acid concentrations were linear.

A blank sample containing 0.5 g of uninfested flour was dispersed in 25 ml of the acetate buffer and stirred for 30 min. at 40°C. After stirring, the sample was centrifuged at 3,000 rpm for 10 min. and the supernatant filtered through a 45-mm AA millipore filter. This solution was used to zero the instrument. Samples of infested flour were read against this blank.

Samples containing 0.5 g of infested flour were dispersed in 25 ml of acetate buffer for the determination of uric acid content. The extraction procedure was exactly as described above for the blank. The uric acid level was determined from the standard curve.

---

[8]P. K. Harien and E. L. Soderstrom, 1966. Coleoptera Infesting Stored Products. C. N. Smith (ed.), Academic Press, New York, p. 241.

Results and Discussion

Fluorescence emission spectra of a solution of uric acid and extracts from infested and uninfested flour with a 390-nm filter provided for the emission monochromator are shown in Figure 1. Use of a 390-nm filter for the emission monochromator avoids interference from the Raman band in aqueous solvents giving rise to large blanks at high sensitivity settings. The 390-nm filter also eliminates scatter peaks from the excitation wavelength, showing only the peak arising from fluorescence.[9]

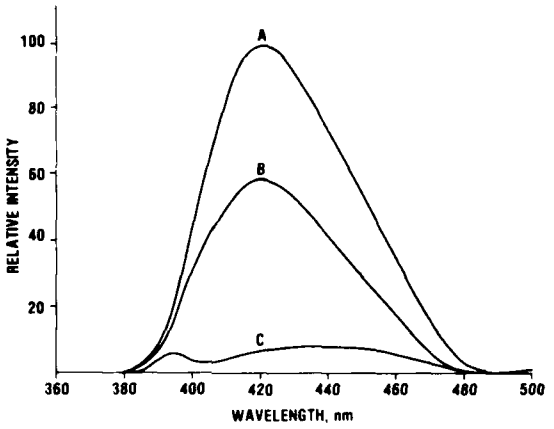

Fig. 1. Fluorescence spectra for solution of uric acid (0.5 mg/ml) (A), extract of uric acid from infested flour (0.3 mg/ml) (B), and extract of uninfested flour (20 mg/ml) (C). Excitation is at 330-nm with an emission maximum (390-nm filter) at 420-nm.

The efficiency of uric acid extraction from flour with 0.2 M sodium acetate was checked by adding known amounts of uric acid to flour. The recovery rate for four flour samples, to each of which was added 8.0 to 40.0 mg/dL of uric acid, is shown in Table 1. The average recovery rate of 96.4% (± 1.4% SD) was satisfactory.

---

[9]C. A. Parker, 1968. Photoluminescence of Solutions. Elsevier Publishing Company, New York, p. 411.

Table 1. Analytical Recoveries of Uric Acid Added to Flour

Sample	Uric Acid (mg/100 g Flour) Added	Found	Recovery
1	8.0	7.7	96.2
2	12.0	11.5	95.8
3	24.0	22.8	95.0
4	48.0	47.3	98.6
Mean Recovery (%±SD)			96.4 ±1.4

In Table 2 are summarized the data for Tribolium. These data show an increase of uric acid content in flour with time and population. The amount of uric acid excreted by Tribolium varies at different stages of its metamorphosis. The larvae produce uric acid at a greater rate than adult insects; however, considerable amounts of uric acid are excreted by the adults and were used in this study for natural insect infestation of flour for the determination of measurable quantities of uric acid.

Table 2. Excretion of Uric Acid by Adult Tribolium[a]

Type of Adult	Days	Uric Acid, mg/100 g of Flour[b] Number of Adult Tribolium 20	40	60
T. castaneum	4	3.0 ± 1.0	4.5 ± 1.5	6.0 ± 2.0
T. confusum	4	2.0 ± 0.5	4.0 ± 1.5	8.0 ± 2.5
T. castaneum	6	7.5 ± 2.0	16.0 ± 4.0	18.0 ± 3.0
T. confusum	6	9.0 ± 1.5	20.0 ± 3.0	22.0 ± 2.0
T. castaneum	8	18.5 ± 3.5	24.0 ± 4.0	28.5 ± 3.0
T. confusum	8	17.0 ± 2.0	27.0 ± 3.0	29.0 ± 5.0

[a]Average of 3 replicate measurements.

[b]Uric acid content in 0.5 g sample of flour from std. curve x 200 = uric acid content in 100 g of test unit.

Infestation by T. confusum and T. costaneum bring about undesirable biochemical changes in flour. Bread prepared from flour infested by Tribolium show a variety of progressive property changes with time. These changes include color, reduction in loaf size, offensive taste and odor.[10] These property changes of flour become more intensified with increasing time of insect infestation.

These non-specific changes indicated by analytical results, relative to insect infestation of flour, are of little value unless this information can be transmitted to predicting storage life and baking qualities. The periodic measuring of the uric acid levels by fluorescence suggests a sensitive quantitative procedure that would be a useful index in predicting the storage potential of flour. In addition, the fluorescence technique would be useful in detecting past infestations of flour where evidence has been removed.

The fluorescence technique would provide necessary supporting data for military medical research and development personnel on the determination of the wholesomeness of insect contaminated flour. On-site evaluation could be provided with inexpensive filter type fluorescence instrumentation which laboratory personnel and/or inspectors could use and evaluate with confidence the utility of stocks which otherwise may have been discarded. The usefulness of such an analytical technique available for the military, from an economic standpoint, would be immeasurable.

Conclusions

These results demonstrate the use of fluorescence in the determination of uric acid content in flour and its correlation with insect infestation. Fluorescence spectroscopy may be carried out with rather simple methodology and instrumentation, allowing laboratory findings to be more easily translated to on-site inspection methods and devices.

The fluorometric technique is a simple, accurate, and sensitive method for the determination or uric acid in flour and would also be applicable for other cereals. The method is capable of detection at a level of 3.0 mg per 100 g of flour. The technique would be extremely advantageous in predicting storage potential of flour and in predicting baking qualities of bread made from infested flour.

---

[10] L. W. Smith, Jr., J. J. Pratt, I. Nii and A. P. Umina, 1971. J. Stored Prod. Res., 6:307.

This document reports research undertaken at the U.S. Army Natick Research and Development Laboratories and has been assigned No. NATICK/TR-81/003 in the series of reports approved for publication.

List of References

Association of Official Analytical Chemists, 1980. Official Methods of Analysis (13th ed.), Method 44:178. The Association, Washington, DC.

Farn, G. and D. M. Smith, 1963. J. Assoc. Off. Anal. Chem., 46:522.

Gupta, P. D. and R. N. Sinha, 1960. Ann. Entomol. Soc. Am., 53:632.

Harien, P. K. and E. L. Soderstrom, 1966. Coleoptera Infesting Stored Products, C. N. Smith (ed.), Academic Press, New York, p. 241.

Katro, S. Ven, R. N. Nuggehalli, S. V. Pringle, M. Swaminathan and V. Subrahmanyan, 1960. Cereal Chem., 37:97.

Kurtz, O. L. and K. L. Harris, 1962. Micro-Analytical Entomology for Sanitation Control, Assoc. Off. Agric. Chem., Washington, DC, p. 13.

Parker, C. A., 1968. Photoluminescence of Solutions, Elsevier Publishing Company, New York, p. 411.

Sen, N. P., 1968. J. Assoc. Off. Anal. Chem., 51:785.

Sen, N. P. and D. M. Smith, 1966. J. Assoc. Off. Anal. Chem., 49:899.

Smith, Jr., L. W., J. J. Pratt, I. Nii and A. P. Umina, 1971. J. Stored Prod. Res., 6:307.